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Synaptic [Ca^{2+}]: Intracellular Stores Spill Their Guts

Minireview

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A critical role of Ca^{2+} in neuronal signaling is to couple electrical excitation to the activation of intracellular enzymes and signal transduction cascades. Presynaptically, action potential–mediated calcium influx releases neurotransmitter from presynaptic terminals. Postsynaptically, calcium ions play a crucial role in the induction of most forms of long-term synaptic potentiation (LTP) and depression (LTD), the putative cellular mechanisms of learning and memory; Ca^{2+} is required to regulate postsynaptic enzymes that trigger rapid modifications of synaptic strength and also to activate transcription factors that facilitate long-term maintenance of these modifications. How can a single second messenger system encode all of these functions with any kind of specificity? The answer is thought to lie in the details: with differences in amplitude, localization, and time course, different Ca^{2+} signals will carry very different biochemical meanings for the cell.

Take, for instance, the case of synaptic plasticity in the CA1 region of the hippocampus: the induction of both LTD and LTP are blocked by postsynaptic chelators of Ca^{2+} and are thus Ca^{2+} dependent. It is hypothesized that features of the intracellular free Ca^{2+} concentration, [Ca^{2+}], produced by the LTD-inducing stimulus differ from those produced by the LTP-inducing stimulus. In some cases, crucial differences could be simply the durations and amplitudes of the stimuli. Yet, the answer may be considerably more complex. For example, it has been shown that CaMKII, a protein kinase critical to synaptic plasticity that is activated by the Ca^{2+}-binding protein calmodulin, can discriminate complex temporal features of the [Ca^{2+}] transient, such as the frequency of [Ca^{2+}] oscillations (De Koninck and Schulman, 1998).

What determines the spatial and temporal pattern of neuronal Ca^{2+} signals? Important factors include Ca^{2+} buffering, diffusion, and extrusion, as well as the dynamic properties of Ca^{2+} sources. Ca^{2+} can enter the cytoplasm from extracellular and intracellular sources via a number of pathways. Excitatory synaptic stimuli and action potentials are transduced into Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (VSCCs). Synaptic currents are partially carried by Ca^{2+} through NMDA receptors (NMDARs) and by some types of AMPARs. Once in the cytoplasm, Ca^{2+} can trigger Ca^{2+}-induced Ca^{2+} release (CICR) from the endoplasmic reticulum (ER) via ryanodine receptors (or IP_3 receptors) (reviewed by Berridge, 1998). A separate route to release from intracellular stores is via activation of metabotropic glutamate receptors that can lead to production of IP_3 and Ca^{2+} release from the ER by IP_3 receptor activation.

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The last decade has seen numerous advances in optical techniques that have allowed the study of neuronal [Ca^{2+}] dynamics with increasing spatial and temporal resolution in relatively intact preparations, including brain slices. Particularly exciting has been the measurement of synaptic [Ca^{2+}] transients in dendrites and spines (Denk et al., 1995; Yuste and Denk, 1995; Koester and Sakmann, 1998; Schiller et al., 1998), not only because of their relevance to synaptic plasticity but also because the small length scales ($\sim$1 μm) and short time scales ($\sim$1 ms) involved test the limits of our tools. These measurements have so far exclusively pointed to influx through glutamate receptors and VSCCs as sources of synaptic Ca^{2+}, but this picture is now beginning to change.

Parallel Fiber–Activated Ca^{2+} Signaling in Purkinje Cell Dendrites

Few Ca^{2+} signaling systems have received as much attention as the dendrites of cerebellar Purkinje cells. These neurons have the largest dendritic arbors of any vertebrate neuron, which are studded with >100,000 dendritic spines, each of which receives input from a single parallel fiber. Parallel fiber–Purkinje cell synapses can undergo LTD when they are repetitively stimulated together with the climbing fiber (reviewed by Linden and Connor, 1995). Purkinje cell dendrites are filled with an extremely elaborate network of ER that extends throughout the cell, even reaching into dendritic spines (Figure 1, top). This ER is studded with large concentrations of IP_3 receptors in dendrites and spines, but ryanodine receptors appear largely restricted to dendritic shafts (Walton et al., 1991). This peculiar anatomy suggests a connection between parallel fiber activity, IP_3-mediated Ca^{2+} release, and perhaps synaptic plasticity.

It has been known for some time that activation of metabotropic glutamate receptors by glutamate application can lead to Ca^{2+} release from intracellular stores in Purkinje cells, pointing to IP_3-mediated Ca^{2+} release from the ER (Llano et al., 1991). In an elegant application of optical uncaging to neurophysiology, Khodakhah and Ogden (1993) directly demonstrated that high concentrations of intracellular IP_3 can liberate Ca^{2+} from intracellular stores in Purkinje cells. More recently, similar experiments have shown that pairing IP_3 release and postsynaptic depolarization can produce LTD at parallel fiber–Purkinje cell synapses (Khodakhah and Armstrong, 1997), supporting the idea that IP_3-mediated Ca^{2+} release is intimately involved with synaptic plasticity. But to cement the connection between the IP_3 pathway and LTD, it had to be shown that afferent stimuli in parallel fibers can in fact produce IP_3-mediated Ca^{2+} signaling. This was achieved convincingly in two papers in a recent issue of *Nature* (Finch and Augustine, 1998; Takechi et al., 1998) that describe IP_3-mediated Ca^{2+} release caused by relatively physiological synaptic stimulation.

Both groups carried out experiments in which a few parallel fibers were stimulated electrically with short bursts (for example, five stimuli at 50 Hz) of pulses while the corresponding synaptic currents were recorded in the soma of a Purkinje cell. The local dendritic [Ca^{2+}]

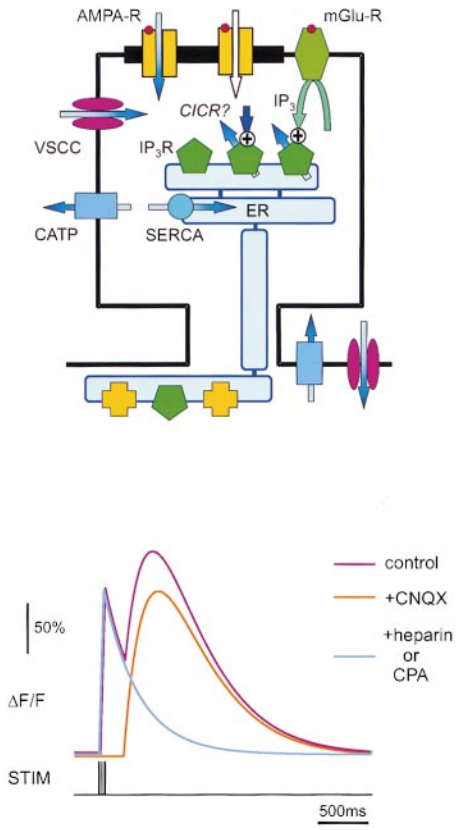

Figure 1. Synaptic Ca^{2+} in Spines of Cerebellar Purkinje Cells

(Top) Schematic representation showing the pathways of Ca^{2+} entry. Blue arrows indicate flow of Ca^{2+}, white arrows indicate flow of current. mGlu-R, metabotropic glutamate receptor; VSCC, voltage-sensitive calcium channel; CATP, Ca^{2+}-ATPase; ER, endoplasmic reticulum; RYR, ryanodine receptor; IP_3R, IP_3 receptor; CICR, Ca^{2+}-induced Ca^{2+} release; SERCA, sarco\endoplasmic reticulum Ca^{2+}-ATPase.

(Bottom) Schematic time course of $[Ca^{2+}]$ transients ($\Delta F/F$ is proportional to $[Ca^{2+}]$) evoked by synaptic stimuli (STIM) in the presence of various drugs (Finch and Augustine, 1998; Takechi et al., 1998).

was measured using a fluorescent Ca^{2+} indicator and confocal microscopy. A burst of synaptic activation produced a biphasic pattern of Ca^{2+} accumulation in the dendritic tree, consisting of a fast component followed by a delayed slow component (Figure 1, bottom). For stimuli activating a large number of parallel fibers, $[Ca^{2+}]$ transients lit up entire dendritic branches, while for weak stimuli transients were localized to individual spines. The time course of the fast component was consistent with depolarization-induced Ca^{2+} influx through VSCCs (during escape from voltage clamp conditions), similar to the $[Ca^{2+}]$ transients previously observed in response to single stimuli (Denk et al., 1995). This component could be selectively abolished by blockade of AMPARs. The slow component, in contrast, could be blocked with drugs that block metabotropic glutamate receptors or IP_3 receptors, or by compounds that deplete intracellular stores; each of these treatments left the fast $[Ca^{2+}]$ transient and synaptic current intact. Therefore, it appears that there are at least two independent mechanisms to couple subthreshold synaptic stimulation to localized dendritic $[Ca^{2+}]$ accumulations. One pathway is triggered by postsynaptic depolarization and leads to opening of VSCCs, while the other is dependent on IP_3 production via metabotropic glutamate receptors. The IP_3 pathway requires repetitive synaptic stimulation because IP_3 concentrations must be at least in the 10 μM range to cause appreciable Ca^{2+} release (Khodakhah and Ogden, 1993), and such high IP_3 concentrations might require accumulation by extended metabotropic glutamate receptor activation (Finch and Augustine, 1998).

Experiments in one of the *Nature* papers (Finch and Augustine, 1998) suggest that IP_3 uncaging alone can induce LTD if repeated for tens of seconds at $\sim$1 Hz. Induction of this type of LTD is restricted to synapses in the vicinity of the site of IP_3 release, with no effect on synapses at a distal site. However, depression by irreversible rundown of synaptic transmission can easily be mistaken for "LTD," and the phototoxic by-products of photolysis could potentially produce such rundown. To settle if IP_3 by itself is sufficient to induce LTD will require further experiments. For example, it remains to be seen if the depression produced by IP_3 uncaging is blocked by IP_3 receptor antagonists, or whether it is occluded by LTD induced in the regular manner.

The precise role of IP_3 in the induction of cerebellar LTD is still an open issue. It is now clear that bursts of activity in parallel fibers produce IP_3 and Ca^{2+} release from intracellular stores. Furthermore, if IP_3 itself can induce LTD, is Ca^{2+} release the only IP_3-mediated effect? If so, what makes $[Ca^{2+}]$ derived from intracellular stores special? In Purkinje cells, signals of similar bulk amplitudes and durations can be produced by complex spikes (Linden and Connor, 1995), but these produce LTD only if paired with parallel fiber stimulation (and presumably IP_3 production). It is possible that IP_3 has as yet unidentified downstream targets that are involved in LTD. Alternatively, it could be that salient features of IP_3-mediated Ca^{2+} release from the ER, such as microdomains of high $[Ca^{2+}]$, are still too fast or too small to be detected by current methods.

Synaptic Ca^{2+} in Hippocampal Pyramidal Neurons

Although the Purkinje cell experiments resolve an important issue, based on previous work the results were not entirely unexpected. For surprising twists, read a paper in the January 1999 issue of *Neuron* reporting on a superficially similar phenomenon in the hippocampus (Emptage et al., 1999). Hippocampal pyramidal cells also have complex dendritic arbors, receiving on the order of 10,000 synapses largely on dendritic spines. These dendrites also contain a complex ER that reaches into a majority of large spines (Figure 2, top). In contrast to the case of Purkinje spines, the ER is studded with ryanodine receptors in dendrites and spines, while IP_3 receptors appear to exist largely in dendritic shafts (Sharp et al., 1993).

The experiments of Emptage et al. were carried out in cultured hippocampal brain slices. A few afferent fibers were stimulated electrically with single stimuli while the corresponding synaptic potentials were recorded in the somata of CA1 or CA3 pyramidal neurons with sharp microelectrodes. Again, local dendritic $[Ca^{2+}]$ was measured using a fluorescent Ca^{2+} indicator and confocal

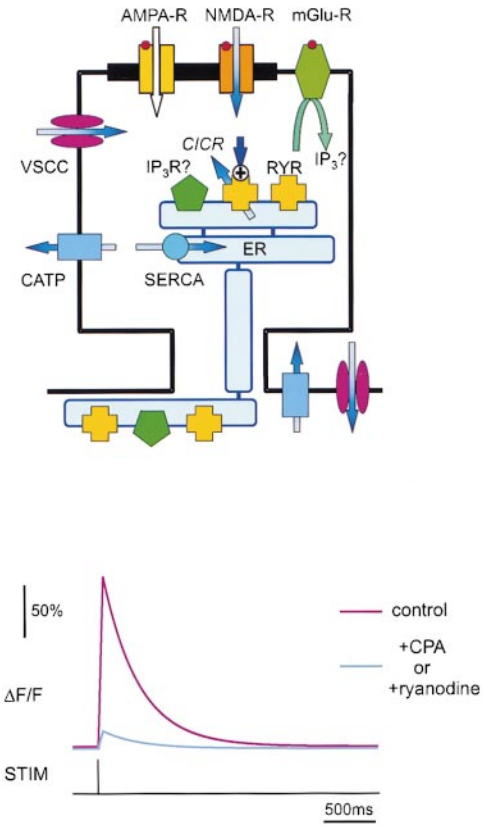

Figure 2. Synaptic Ca^{2+} in Spines of CA1 Hippocampal Pyramidal Neurons

(Top) Schematic representation showing the pathways of Ca^{2+} entry. Note that only a subpopulation of spines contain ER. For abbreviations, see the Figure 1 legend. Some IP_3 receptors may also exist in CA1 spines (Sharp et al., 1993).

(Bottom) Schematic time course of $[Ca^{2+}]$ transients (ΔF/F) evoked by synaptic stimuli (STIM) in the presence and absence of blockers of CICR (Emptage et al., 1999).

microscopy. Consistent with previous work in hippocampus (Yuste and Denk, 1995) and neocortex (Koester and Sakmann, 1998), fast synaptically evoked $[Ca^{2+}]$ transients could be observed localized to individual spines (Figure 2, bottom). Emptage et al. were able to dramatically reduce the amplitudes of these transients by application of CNQX, consistent with a scenario in which depolarization provided by AMPARs opens VSCCs or relieves the magnesium block of NMDARs to allow Ca^{2+} influx. Spine $[Ca^{2+}]$ transients were also blocked by the NMDAR antagonist APV without affecting the amplitude of synaptic potentials, implying that influx through VSCCs is insignificant under these conditions. The simplest explanation for these observations is that the source of intracellular $[Ca^{2+}]$ is influx through NMDARs (as suggested by Koester and Sakmann, 1998). Consistent with this hypothesis, blocking AMPAR-mediated currents in the absence of Mg^{2+} had no effect on $[Ca^{2+}]$ transients, even though synaptic potentials were almost completely abolished; in the absence of Mg^{2+}, AMPA currents were not required since NMDAR conductance no longer requires depolarization. But when Emptage et al. interfered with CICR, the house of cards came crashing down. They used both ryanodine, which locks the ryanodine receptor open, and cyclopiazonic acid (CPA), which blocks Ca^{2+}-ATPases and thus depletes intracellular stores: both treatments almost completely abolished synaptically evoked $[Ca^{2+}]$ transients (Figure 2, bottom). With CICR blocked, residual Ca^{2+} influx (now presumably through NMDARs) was so small as to be detectable only during trains of synaptic stimuli.

What then is the role of NMDARs? It is possible that the tiny Ca^{2+} current carried by NMDARs is sufficient to trigger CICR. However, activation of ryanodine receptors typically requires large $[Ca^{2+}]$ (~1 μM), incompatible with the small bulk NMDAR-mediated $[Ca^{2+}]$ signals Emptage et al. measured. Therefore, ryanodine receptors would have to be located very close to the conduction pores of NMDARs and their microdomains of high $[Ca^{2+}]$. Although this may seem exotic, the Ca^{2+} release units of cardiac muscle, consisting of L-type VSCCs and ryanodine receptors, present a precedent for a similar sort of arrangement (Franzini-Armstrong, 1996).

The results by Emptage et al. are likely to fan the flames of controversy. For example, a recent paper on spine $[Ca^{2+}]$ transients in neocortial pyramidal cells evoked by glutamate uncaging found that spine Ca^{2+} influx was in large part due to VSCCs (Schiller et al., 1998) and therefore not NMDAR-mediated CICR. Other recent studies on neocortical (Koester and Sakmann, 1998) and hippocampal (Yuste et al., 1999) pyramidal cells demonstrated boosting of NMDAR-mediated spine $[Ca^{2+}]$ transients when followed by a back-propagating action potential. This result makes sense in terms of the voltage sensitivity of the NMDAR conductance but is hard to reconcile with the NMDAR-dependent CICR reported by Emptage et al. One would require that the CICR $[Ca^{2+}]$ transient be smoothly graded, which would be somewhat surprising for such a positive-feedback system. These four studies lead to differing conclusions about the source of synaptic calcium and are hard to reconcile; all appear technically solid, with a variety of control experiments neatly in place. We can only speculate that the discrepancies may derive from some of the differences in preparation (acute neocortical brain slices versus cultured hippocampal brain slices, whole-cell recording versus sharp-electrode recording, synaptic stimulation versus glutamate uncaging) or other experimental differences. Additional complications could arise because of heterogeneous pathways of Ca^{2+} influx in different spines. For example, Ca^{2+}-permeable AMPA receptors appear to exist in a small subset of Purkinje (Denk et al., 1995) and CA1 (Yuste et al., 1999) spines. Differences in results between different studies could thus be in part due to different spine selection criteria.

A question soon to be burning in many minds is what role NMDAR-mediated CICR might play in the induction of synaptic plasticity. Ca^{2+} stores have been implicated in the induction of synaptic plasticity in only a handful of studies. For example, in CA1, LTP has been blocked by depleting internal stores with thapsigargin (Harvey and Collingridge, 1992). Also in CA1, LTD was prevented in one study by intracellular blockade of IP_3 receptors (but *not* ryanodine receptors) (Reyes and Stanton, 1996). In dentate gyrus, ryanodine receptors were found to participate in the induction of NMDAR-*independent*

forms of LTP (Wang et al., 1996) (but *not* in NMDAR-dependent LTP). Future research will need to investigate the nature and relative contribution of CICR to [Ca^{2+}] dynamics under different stimulus protocols (in particular, those that are known to induce plasticity). It might be that CICR is only obligatory for plasticity under conditions of weak NMDAR activation.

Conclusions

The canonical view holds that at synapses neurotransmitters act postsynaptically via conductance changes. At the parallel fiber–Purkinje cell synapse it is now clear that synaptic activation of metabotropic glutamate receptors can produce postsynaptic Ca^{2+} release from intracellular stores without postsynaptic currents. Similarly, in the hippocampus, synaptic NMDAR activation can produce postsynaptic Ca^{2+} release from ryanodine-sensitive stores. Much work remains to be done to clarify the mechanisms and to define the functions of these synaptic events. These functions will almost certainly be tied to the distinct spatiotemporal characteristics of the [Ca^{2+}] dynamics produced by release from intracellular stores.

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